FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 50179-088

U.S. APPLIC. NO. (if known, see 37 CFR 1.5): 09/807519

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU99/00896	October 18, 1999	October 16, 1998

TITLE OF INVENTION

DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN

APPLICANT(S) FOR DO/EO/US

Mitchell KEEGAN, Mark Richard JONES, and Geoffrey Philip M. MOORE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau. (A copy of the published application is transmitted herewith.)
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendment has NOT expired
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information.

International Search Report
International Preliminary Examination Report

20277

PATENT TRADEMARK OFFICE

U.S. APPLIC. NO. (if known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/807519	PCT/AU99/00896	50179-088

	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,000.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	27 -20 =	7	x $18.00	$ 126.00	
Independent Claims	2 -3 =	0	x $80.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
			TOTAL OF ABOVE CALCULATIONS =	$ 1,256.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
			SUBTOTAL =	$ 1,256.00	
Processing fee of $130.00 for furnishing the English translation later than the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$	
			TOTAL NATIONAL FEE =	$ 1,256.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property			+	$	
			TOTAL FEES ENCLOSED =	$ 1,256.00	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 500417 in the amount of $ 1,256.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 500417. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

McDERMOTT, WILL & EMERY
600 13th Street, N.W.
Washington, DC 20005-3096
(202) 756-8000
Facsimile (202) 756-8087

SIGNATURE	Robert L. Price
Robert L. Price	
NAME	
22,685	
REGISTRATION NUMBER	
April 16, 2001	
DATE	

BASED ON FORM PTO-1390 (Rev. 5-93)

Docket No.: 50179-088 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Mitchell KEEGAN, et al.	:	
Serial No.:	:	Group Art Unit:
Filed: April 16, 2001	:	Examiner:

For: DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination of the above-referenced application, please amend the application as follows:

IN THE CLAIMS, DRAWINGS, AND SEQUENCE LISTING:

Please substitute the attached amended pages of claims, drawings, and sequence listing for the corresponding pages as filed herewith.

IN THE CLAIMS (as amended):

Claim 4, line 1, please change "any one of claims 1 to 3" to --claim 1--.

Claim 7, line 1, please change "any of claims 1 to 6" to --claim 1--.

Claim 8, lines 1 and 2, please change "any one of claims 1 to 7" to --claim 1--.

Claim 9, line 2, please change "any one of claims 1 to 7" to --claim 1--.

Claim 13, line 2, please change "any one of claims 9 to 12" to --claim 9--.

Claim 14, lines 2 and 3, please change "any one of claims 9 to 12" to --claim 9--.

Claim 16, line 3, please change "any one of claims 1 to 7" to --claim 1--.

Claim 17, lines 2 and 3, please delete "or 15".

Claim 18, line 1, please delete "or 17".

Claim 24, line 1, please change "any one of claims 21 to 23" to --claim 21--.

Claim 26, line 1, please change "any one of claims 21 to 25" to --claim 21--.

Claim 27, line 1, please change "any one of claims 21 to 26" to --claim 21--.

REMARKS

The above-referenced application is amended to delete the multiple dependency of claims 4, 7-9, 13-14, 16-18, 24, and 26-27 to avoid the multiple dependent claim filing fee.

Respectfully submitted,

MCDERMOTT, WILL & EMERY

Robert L. Price
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Date: April 16, 2001
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FIGURE 1: ISS-pST gene construct

```
   1  GCTAGCATGG CCCTGTGGAT GCGCCTCCTG CCCCTGCTGG CGCTGCTGGC
  51  CCTCTGGGGA CCTGACCCAG CCGCAGCCCT CGAGATGTTT CCAGCTATGC
 101  CACTTTCTTC TCTGTTCGCT AACGCTGTTC TTCGGGCCCA GCACCTGCAC
 151  CAACTGGCTG CCGACACCTA CAAGGAGTTT GAGCGCGCCT ACATCCCGGA
 201  GGGACAGAGG TACTCCATCC AGAACGCCCA GGCTGCCTTC TGCTTCTCGG
 251  AGACCATCCC GGCCCCCACG GGCAAGGACG AGGCCCAGCA GAGATCGGAC
 301  GTGGAGCTGC TGCGCTTCTC GCTGCTGCTC ATCCAGTCGT GGCTCGGGCC
 351  CGTGCAGTTC CTCAGCAGGG TCTTCACCAA CAGCCTGGTG TTTGGCACCT
 401  CAGACCGCGT CTACGAGAAG CTGAAGGACC TGGAGGAGGG CATCCAGGCC
 451  CTGATGCGGG AGCTGGAGGA TGGCAGCCCC CGGGCAGGAC AGATCCTCAA
 501  GCAAACCTAC GACAAATTTG ACACAAACTT GCGCAGTGAT GACGCGCTGC
 551  TTAAGAACTA CGGGCTGCTC TCCTGCTTCA AGAAGGACCT GCACAAGGCT
 601  GAGACATACC TGCGGGTCAT GAAGTGTCGC CGCTTCGTGG AGAGCAGCTG
 651  TGCCTTCTAG TCTAGA  (SEQ ID NO:4)
```

ATG...GCC- insulin secretory signal.

GCTAGC- *Nhe* I restriction site incorporated into construct in order to ligate into plasmid.

CTCGAG- *Xho* I restriction site incorporated into construct in order to ligate secretory signal and pST.

TCTAGA- *Xba* I restriction site incorporated into construct in order to ligate into plasmid.

FIGURE 2: ISS-pST peptide sequence.

```
  1  MALWMRLLPL LALLALWGPD PAAALEMFPA MPLSSLFANA VLRAQHLHQL
 51  AADTYKEFER AYIPEGQRYS IQNAQAAFCF SETIPAPTGK DEAQQRSDVE
101  LLRFSLLLIQ SWLGPVQFLS RVFTNSLVFG TSDRVYEKLK DLEEGIQALM
151  RELEDGSPRA GQILKQTYDK FDTNLRSDDA LLKNYGLLSC FKKDLHKAET
201  YLRVMKCRRF VESSCAF    (SEQ ID NO:3)
```

MAL....AAA- insulin secretory signal, cleaved upon secretion of pST.
LE- function of XhoI cleavage site; result in no predicted secondary structural changes to pST.

Sequence listing:

Applicants: Commonwealth Scientific and Industrial Research Organisation
University of Western Sydney (Nepean)
Pig Research and Development Corporation

Title of the Invention: Delivery system for porcine somatotropin

Prior Application Number: PP 6556
Prior Application Filing Date: 1998-10-16

Number of SEQ ID NOs: 4

Software: PatentIn Ver. 2.1

SEQ ID NO: 1
Length: 24
Type: PRT
Organism: Homo sapien

Sequence: 1
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15

Trp Gly Pro Asp Pro Ala Ala Ala
20

SEQ ID NO: 2
Length: 72
Type: DNA
Organism: Homo sapien

Sequence: 2

atggccctgt ggatgcgcct cctgcccctg ctggcgctgc tggccctctg gggacctgac 60
ccagccgcag cc

SEQ ID NO: 3

Length: 666

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: ISS-pST gene construct

Sequence: 3

gctagcatgg ccctgtggat gcgcctcctg cccctgctgg cgctgctggc cctctgggga 60
cctgacccag ccgcagccct cgagatgttt ccagctatgc cactttcttc tctgttcgct 120
aacgctgttc ttcgggccca gcacctgcac caactggctg ccgacaccta caaggagttt 180
gagcgcgcct acatcccgga gggacagagg tactccatcc agaacgccca ggctgccttc 240
tgcttctcgg agaccatccc ggcccccacg ggcaaggacg aggcccagca gagatcggac 300
gtggagctgc tgcgcttctc gctgctgctc atccagtcgt ggctcgggcc cgtgcagttc 360
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ctgaaggacc tggaggaggg catccaggcc ctgatgcggg agctggagga tggcagcccc 480
cgggcaggac agatcctcaa gcaaacctac gacaaatttg acacaaactt gcgcagtgat 540
gacgcgctgc ttaagaacta cgggctgctc tcctgcttca agaaggacct gcacaaggct 600
gagacatacc tgcgggtcat gaagtgtcgc cgcttcgtgg agagcagctg tgccttctag 660
tctaga 666

SEQ ID NO: 4

Length: 217

Type: PRT

Organism: Artificial Sequence

Feature:
Other Information: Description of Artificial Sequence: ISS-pST peptide sequence

Sequence: 4

```
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1               5                  10                  15

Trp Gly Pro Asp Pro Ala Ala Ala Leu Glu Met Phe Pro Ala Met Pro
            20                  25                  30

Leu Ser Ser Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His
        35                  40                  45

Gln Leu Ala Ala Asp Thr Tyr Lys Glu Phe Glu Arg Ala Tyr Ile Pro
    50                  55                  60

Glu Gly Gln Arg Tyr Ser Ile Gln Asn Ala Gln Ala Ala Phe Cys Phe
65                  70                  75                  80

Ser Glu Thr Ile Pro Ala Pro Thr Gly Lys Asp Glu Ala Gln Gln Arg
                85                  90                  95

Ser Asp Val Glu Leu Leu Arg Phe Ser Leu Leu Leu Ile Gln Ser Trp
            100                 105                 110

Leu Gly Pro Val Gln Phe Leu Ser Arg Val Phe Thr Asn Ser Leu Val
        115                 120                 125

Phe Gly Thr Ser Asp Arg Val Tyr Glu Lys Leu Lys Asp Leu Glu Glu
    130                 135                 140

Gly Ile Gln Ala Leu Met Arg Glu Leu Glu Asp Gly Ser Pro Arg Ala
145                 150                 155                 160
```

Gly Gln Ile Leu Lys Gln Thr Tyr Asp Lys Phe Asp Thr Asn Leu Arg
165 170 175

Ser Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Ser Cys Phe Lys
180 185 190

Lys Asp Leu His Lys Ala Glu Thr Tyr Leu Arg Val Met Lys Cys Arg
195 200 205

Arg Phe Val Glu Ser Ser Cys Ala Phe
210 215

Claims:

1. An expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

2. An expression cassette according to claim 1, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

3. An expression cassette according to claim 1, wherein the insulin secretory signal is a modified insulin secretory signal comprising modifications of the insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1, wherein said modifications do not deleteriously affect the biological activity of the insulin secretory signal.

4. An expression cassette according to any one of claims 1 to 3, wherein the heterologous sequence encodes a polypeptide selected from hormones, cytokines, receptor agonists, receptor antagonists, pheromones, and enzymes.

5. An expression cassette according to claim 4, wherein the polypeptide is a growth hormone.

6. An expression cassette according to claim 5, wherein the polypeptide is somatotropin.

7. An expression cassette according to any of claims 1 to 6, further including one or more regulatory elements to enable pulsatile expression of the heterologous sequence .

8. A vector including an expression cassette according to any one of claims 1 to 7.

9. A recombinant cell which includes an expression cassette according to any one of claims 1 to 7.

10. A recombinant cell according to claim 9, wherein the cell is a bacterial, yeast, insect or mammalian cell.

21. A method of administering somatotropin to a pig, wherein the method includes implanting in the pig a capsule including a semi-permeable membrane encapsulating recombinant cells, said recombinant cells including and expressing an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding somatotropin, wherein said membrane is permeable to the expresssed somatotropin.

22. A method according to claim 21, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

23. A method according to claim 21, wherein the insulin secretory signal is a modified insulin secretory signal comprising modifications of the insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1, wherein said modifications do not deleteriously affect the biological activity of the insulin secretory signal.

24. A method according to any one of claims 21 to 23, wherein the recombinant cells are mammalian cells.

25. A method according to claim 24, wherein the mammalian cells are rat myoblast (L6) cells.

26. A method according to any one of claims 21 to 25, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

27. A method according to any one of claims 21 to 26, wherein the pig is implanted with one or more capsules sufficient to achieve secretion of somatotropin of at least 30 ng/ml.

Attorneys' Docket No.: 50179-088 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Mitchell KEEGAN, et al.	)	
	)	Group Art Unit: TBA
Serial No.: 09/807,519	)	
	)	Examiner: TBA
Filed: April 16, 2001	)	
	)	
For: DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN	)	

REPLY TO NOTIFICATION TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Notification To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/or Amino Acid Sequence Disclosures dated May 17, 2001, please amend the above-captioned application as follows.

IN THE SPECIFICATION

Please replace the first full paragraph on page 5 of the specification with the following paragraph in its place:

--Figure 1: (SEQ ID NO:2 and SEQ ID NO: 4) Insulin secretory signal - pST gene construct.--

Please replace the second full paragraph on page 5 of the specification with the following paragraph in its place:

--Figure 2: (SEQ ID NO: 1 and SEQ ID NO: 3) Insulin secretory signal - pST peptide sequence.--

Please insert at the end of the specification, the attached paper copy of a Sequence Listing.

REMARKS

In response to the Notification, Applicants submit a Sequence Listing both in paper and computer readable form in full compliance with the Sequence Rules as set forth in 37 C.F.R. § 1.821 through 1.825.

In accordance with 37 C.F.R. § 1.821(f) the undersigned representative hereby states that the content of the Sequence Listing of the attached paper copy of the Sequence Listing of the above-captioned application and the computer readable copy filed herewith on a computer-readable disk are believed to be the same. Moreover, the paper copy and above-referenced computer readable copy do not introduce new matter into the application.

Prompt and favorable consideration on the merits of the application is respectfully requested.

Applicants respectfully request any extension of time deemed necessary. If necessary, please also charge any deficient fees, or credit any overpayment of fees, to Deposit Account No. 500417. A duplicate copy of this communication is enclosed.

[SIGNATURE PAGE TO FOLLOW]

Respectfully submitted,

MCDERMOTT, WILL & EMERY

By: 
Kelli N. Watson
Registration No. 47,170

September 17, 2001

McDermott, Will & Emery
600 Thirteenth Street, N.W.
Washington, D.C. 20005-3096
Telephone: (202) 756-8351
Facsimile: 202) 756-8087

Attachment: Paper Copy of a Sequence Listing
Computer Readable Form of a Sequence Listing

ATTACHMENT

Version With Markings To Show Changes Made

IN THE SPECIFICATION:

Figure 1: (SEQ ID NO:2 and SEQ ID NO: 4) Insulin secretory signal - pST gene construct.

Figure 2: (SEQ ID NO: 1 and SEQ ID NO: 3) Insulin secretory signal - pST peptide sequence.

SEQUENCE LISTING

<110> Keegan, Mitchell

Moore, Geoffrey Philip M.

Jones, Mark Richard

<120> Delivery System For Porcine Somatotropin

<130> 050179-0088

<140> 09/807,519

<141> 2001-04-16

<150> PCT/AU99/00896

<151> 1999-10-18

<150> PP 6556

<151> 1998-10-16

<160> 4

<170> PatentIn version 3.1

<210> 1

<211> 24

<212> PRT

<213> Homo Sapien

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1 5 10 15

Trp Gly Pro Asp Pro Ala Ala Ala
20

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ccagccgcag cc 72

<210> 3

<211> 217

<212> PRT

<213> Artificial Sequence

<220>

<223> Unknown Organsim

<400> 3

Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15

Trp Gly Pro Asp Pro Ala Ala Ala Leu Glu Met Phe Pro Ala Met Pro
20 25 30

Leu Ser Ser Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His
35 40 45

Gln Leu Ala Ala Asp Thr Tyr Lys Glu Phe Glu Arg Ala Tyr Ile Pro
50 55 60

Glu Gly Gln Arg Tyr Ser Ile Gln Asn Ala Gln Ala Ala Phe Cys Phe
65 70 75 80

Ser Glu Thr Ile Pro Ala Pro Thr Gly Lys Asp Glu Ala Gln Gln Arg
85 90 95

Ser Asp Val Glu Leu Leu Arg Phe Ser Leu Leu Leu Ile Gln Ser Trp
100 105 110

Leu Gly Pro Val Gln Phe Leu Ser Arg Val Phe Thr Asn Ser Leu Val
115 120 125

Phe Gly Thr Ser Asp Arg Val Tyr Glu Lys Leu Lys Asp Leu Glu Glu
130 135 140

Gly Ile Gln Ala Leu Met Arg Glu Leu Glu Asp Gly Ser Pro Arg Ala
145 150 155 160

Gly Gln Ile Leu Lys Gln Thr Tyr Asp Lys Phe Asp Thr Asn Leu Arg
165 170 175

Ser Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Ser Cys Phe Lys
180 185 190

Lys Asp Leu His Lys Ala Glu Thr Tyr Leu Arg Val Met Lys Cys Arg
195 200 205

Arg Phe Val Glu Ser Ser Cys Ala Phe
210 215

<210> 4

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<212> DNA

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<223> Unknown Organism

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cctgacccag ccgcagccct cgagatgttt ccagctatgc cactttcttc tctgttcgct 120

aacgctgttc ttcgggccca gcacctgcac caactggctg ccgacaccta caaggagttt 180

gagcgcgcct acatcccgga gggacagagg tactccatcc agaacgccca ggctgccttc 240

tgcttctcgg agaccatccc ggcccccacg ggcaaggacg aggcccagca gagatcggac 300

gtggagctgc tgcgcttctc gctgctgctc atccagtcgt ggctcgggcc cgtgcagttc 360

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ctgaaggacc tggaggaggg catccaggcc ctgatgcggg agctggagga tggcagcccc 480

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gagacatacc tgcgggtcat gaagtgtcgc cgcttcgtgg agagcagctg tgccttctag 660

tctaga 666

DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN

Field of the Invention:

The present invention relates to an expression construct for delivering an exogenous polypeptide to a host. The present invention also relates to recombinant cells which include this expression construct and to semi-permeable capsules which include the recombinant cells.

Background of the Invention:

In mammals, somatotropin (growth hormone) is normally secreted from the pituitary gland. However, exogenous administration of somatotropin to pigs has been shown to improve feed efficiency 15-20%, increase daily weight gain 10-15%, reduce carcass fat 10-20%, increase lean meat content 5-10% and reduce feed intake. Unfortunately, somatotropin (which is a small protein of 190 amino acids) is susceptible to gastric acids and protein digestion hence daily injections are required in order to be efficacious. Currently, welfare and ethical issues discourage the use of the pneumatic pST injection gun and the costs of daily administration restrict industry-wide adoption.

Recent advances in gene therapy have enabled the development of strategies which avoid the dependence on autologous target cells and immunosuppressive therapy by utilising transfected cells encapsulated in a semi-permeable alginate-poly-L-lysine-alginate (APA) membrane. The APA capsule environment is compatible with cell viability and growth so that transfected cells remain viable, secreting growth factors, for extended periods. The APA is permeable to small proteins and consequently gene expression can be controlled by external means. The APA barrier inhibits immune surveillance and cell rejection events so that non-host, highly expressing, cells can be employed in the capsule. The APA barrier may also prevent uncontrolled proliferation of the transfected cells in the recipient host. The APA capsule can be removed, potentially re-used, in order to negate the concerns regarding consumption of transgenic material. Further, if the capsule is damaged by severe tissue trauma a normal host-graft rejection would destroy the implanted cells.

Summary of the Invention:

The present inventors have now found that ligation of an insulin secretory signal to a heterologous gene sequence prior to introduction of the gene sequence into a host cell results in a surprising increase in the level of secretion of the heterologous gene product. This finding has led to the development of an improved gene delivery system involving encapsulation of recombinant cells for implantation into a host.

Accordingly, in a first aspect, the present invention provides an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

By "heterologous sequence" we mean a sequence other than a sequence encoding insulin.

By "operably linked" we mean that the insulin secretory signal sequence is contiguous and in reading frame with the heterologous coding sequence.

The preferred insulin secretory signal is an insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1. However, it will be appreciated by those skilled in the art that a number of modifications may be made to that secretory signal without deleteriously affecting the biological activity of the signal. For example, this may be achieved by various changes, such as sulfation, phosphorylation, nitration and halogenation; or by amino acid insertions, deletions and substitutions, either conservative or non-conservative (eg. D-amino acids, desamino acids) in the peptide sequence where such changes do not deleteriously affect the overall biological activity of the secretory signal. Thus, the inclusion in the expression cassette of an insulin secretory signal which has been modified in one or more of the abovementioned ways, is to be regarded as being encompassed by the present invention.

The heterologous sequence may encode any polypeptide, other than insulin, of interest. For example, the heterologous sequence may encode a hormone, cytokine, receptor agonist or antagonist, pheromone or enzyme. In a preferred embodiment, the heterologous sequence encodes a growth hormone. Preferably, the growth hormone is somatotropin.

In a second aspect, the present invention provides a vector including an expression cassette of the first aspect. The vector may be any suitable

vector for introducing the expression cassette into a cell. Suitable vectors include viral vectors and bacterial plasmids.

The expression cassette of the first aspect of the present invention, or the vector of the second aspect, may further include one or more elements which regulate gene expression. Examples of suitable regulatory elements include the Melatonin Response Element (MRE) (as described in Schrader *et al, 1996*, the entire contents of which are incorporated herein by reference), and/or rapamycin mediated transcription factors (as described in Magari *et al, 1997*, the entire contents of which are incorporated herein by reference). In a preferred embodiment, the regulatory element(s) enable pulsatile expression of the polypeptide of interest.

In a third aspect, the present invention provides a recombinant cell which includes an expression cassette according to the first aspect of the present invention.

The recombinant cell may be a bacterial, yeast, insect or mammalian cell. In a preferred embodiment, the recombinant cell is a mammalian cell. In a further preferred embodiment, the cell is a rat myoblast (L6) cell.

In a fourth aspect, the present invention provides a method of producing a polypeptide which includes culturing a recombinant cell of the third aspect under conditions enabling the expression and secretion of the polypeptide and optionally isolating the polypeptide.

The recombinant cell(s) of the present invention may be encapsulated in a semi-permeable matrix for delivery or implantation in a host.

Accordingly, in a fifth aspect, the present invention provides a capsule for implantation in a host, the capsule including a semi-permeable membrane which encapsulates one or more recombinant cells according to the third aspect of the present invention.

In a preferred embodiment, the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane. The preparation of an APA semi-permeable membrane is described in Basic *et al, 1996*, the entire contents of which are incorporated herein by reference.

In a sixth aspect, the present invention provides a method of administering a polypeptide to a host which includes administering to the host an expression cassette according to the first aspect of the present invention.

In a seventh aspect, the present invention provides a method of administering a polypeptide to a host which includes implanting in the host a capsule according to the fifth aspect of the present invention.

The host may be any animal or human. In a preferred embodiment, the host is a livestock animal. In a further preferred embodiment, the host is selected from the group consisting of grazing cattle, feed-lot cattle, dairy cows, pigs and poultry.

It will be appreciated by those skilled in the art that the present invention provides an improved system for the delivery of genetic material to a host. The ligation of the insulin secretory signal to a biologically active polypeptide leads to increased secretion of the polypeptide from recombinant cells. Following secretion, the secretory signal may be cleaved leaving the biologically active polypeptide. The recombinant cells, when encapsulated in a semi-permeable membrane, have the capacity to secrete significant amounts of the biologically active polypeptide and the semi-permeable membrane enables control of gene expression by external means. Implantation of the encapsulated recombinant cells provides an advantage in that the implantation requires minimal surgery. Further, the semi-permeable membrane reduces immune surveillance and cell rejection which means that non-host cells can be employed in the capsule.

In a preferred embodiment, the semi-permeable membrane is durable which provides an advantage in that it may limit cell growth thereby preventing uncontrolled proliferation in the recipient host. The capsules provide a further advantage in that they may be removed and re-used.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following non-limiting Examples and Figures.

Brief description of the accompanying figures:

Figure 1: Insulin secretory signal - pST gene construct.

Figure 2: Insulin secretory signal - pST peptide sequence.

Figure 3: Rate of weight gain (from day 0) for control and individual pST-L6IXS treated pigs.

Figure 4: Percentage weight gain for control and individual pST-L6IXS treated animals.

Figure 5: Plasma, pST levels for control and individual pST-L6IXS treated animals.

Figure 6: Plate 1- Appraisal of pST-L6IXS capsule administration site
Plate 2 - Placement of pST-L6IXS capsule in culture media for ex-vivo assessment.

Figure 7: Ex-vivo assessment of secretion of pST from capsules for a 24 hr period following removal from host animal.

Figure 8: Mean plasma pST (over 3 hours @ 30 min intervals) before (white bars) and 1 week post pST capsule administration (black bars) (*significant).

Figure 9: Daily plasma pST concentrations of two pigs, pig 206 and 228, with implanted capsules secreting 25 ng/ml and 500 ng/ml respectively.

Figure 10: Rate of Gain (ROG) in kg/day (black squares) and P2 back fat measurements in pigs produced in Example 4.

Figure 11: Rate of Gain (ROG) of male pigs following implantation with pST secreting or control immunoneutral gene therapy (IGT) capsules (± SEM).

Figure 12: Back fat (P2) of male pigs following implantation with pST secreting or control immunoneutral gene therapy (IGT) capsules (± SEM).

Figure 13: Loin (eye) muscle area of male pigs following implantation with pST secreting or control immunoneutral gene therapy (IGT) capsules (± SEM).

Detailed description of the invention:

Example 1: Cloning of the ISS-pST construct

The pST gene was obtained from Southern Cross Biotechnology Pty Ltd in an *E. coli* bacterium. The plasmid containing the pST gene, pMG939, was isolated from the bacterium using standard plasmid preparation techniques. The PCR primers were designed to amplify the pST gene, add an *Xho* I site to the 5' end and an *Xba* I site to the 3' end to enable ligation events.

The modified pST gene sequence was subsequently ligated to a secretory signal sequence (ISS) derived from the preproinsulin cDNA. *Nhe* I (GCTAGC) and *Xba* I (TCTAGA) restriction sites were constructed in front of the ISS start codon and after the 3' terminal codon of pST, respectively, to allow incorporation into the pCI-neo plasmid (Promega). The pST fusion construct was subsequently isolated and sequenced to verify the coding region (Figure 1).

Transfection of rat myoblast (L6) cells (pST gene incorporation into cells) was performed, with LipoTAXI (Stratagene), 2hrs after the L6 cells were trypsin treated. pST transfected L6 cell clones were maintained in culture, selected with G418, until $>10^7$ cells were generated. Aliquots (2ml) of the culture supernatant were stored at -20°C prior to assessment of pST concentrations in a pST radioimmunoassay (RIA) established by Dr P. Wynn at Sydney University (Camden). The RIA sensitivity was deemed to be >0.4ng/ml with CV's in the order of 12.4%. The polyclonal antisera was raised in guinea pigs with a pST peptide antigen. The RIA results (Table 1) indicate that the pST gene construct produced protein (Figure 2) which is recognised by polyclonal antisera raised against the native form of pST, purified from porcine pituitary glands. L6 Clones pCI/pst-1..5 were generated from the modified transfection technique as described below.

Modified transfection protocol

Characteristically, L6 cells adhere to culture plates and require detachment with trypsin to passage cells; transfection is routinely performed 24hrs later. This procedure resulted in L6 cell clones (n=10) secreting pST at 6-18 ng/ml. Applying LipoTAXI (Promega) and the ISS/pST plasmid to the L6 cells 2hrs after trypsin treatment increased the secretion rate of pST 10-20 fold (>180ng/ml, n=5 clones). This higher pST secretion rates reduce the number of cells (capsules) required to enhance growth.

TABLE 1: Concentrations (ng/ml) for each clone transfected with ISS-pST.

L6 clone	pST (ng/ml)
pCI/pst-1*	182
pCI/pst-2*	188
pCI/pst-3*	188
pCI/pst-4*	140
pCI/pst-5*	200
pCI/pst-6	17
pCI/pst-7	12
pCI/pst-8	8
pCI/pst-9	9
pCI/pst-10	7
pCI/pst-11	7
pCI/pst-12	10
pCI/pst-13	8
pCI/pst-14	6
pCI/pst-15	18

Example 2: Preparation of the porcine somatotropin-rat myoblast (L6) immunoneutral expression system (pST-L6IXS)

The encapsulation procedure described in Basic *et al, 1996*, was followed with the following modifications.

Encapsulation of cells at room temperature, utilises calcium chloride (or lactate) [100mM] to gel the alginate [1.5% w/v] droplets followed immediately by washing with saline (0.9% NaCl) then resuspending in poly-L-lysine [0.05%] for 5 min. Calcium chloride crosslinking for 10min at 37°C resulted in an alginate matrix that was more compatible with cell viablity.

After the poly-L-lysine coating and saline washes another alginate layer is added. Sodium citrate [55mM] treatment for 4min at room temperature softens the capsule to a consistency that increases the difficulty of further manipulation. Cell viablity is apparently reduced to <35% with 4 min exposure to sodium citrate. Placing the capsules in a cell strainer prior to sodium citrate treatment enabled 1min exposure, at 37°C, improving cell viability to >98%.

Procedural and equipment modifications to the encapsulation protocol improved the efficiency (time and resources) of encapsulation with routine increases in cell viability in the order of 64%.

Example 3: Pilot experiment (1) involving implantation of pST-L6IXS in pigs

Preliminary results obtained with the pST-L6IXS, administered to growing mice, indicate enhanced growth characteristics. In a pilot experiment with male pigs (n=9, mean live weight 61 kg) varying numbers of pST-L6IXS were administered in different sites (3 capsules, i.m. in the neck muscle, 3 capsules s.c. in the neck, 10 capsules s.c. at the base of the ear, 20 capsules i.m. in the neck or 29 capsules i.m. in the neck of individual animals on day 0). Blood samples (10ml) were collected via jugular venipuncture and P2 ultra-sound (us) measurements were recorded at -14, 0, 7, 14, 21, 28 and 36 days post administration. The sites of pST-L6IXS administration were monitored for tissue reaction events throughout the experiment. On day 36 animals were euthanased and carcass analysis (back fat depth, BF(mm); eyemuscle area, EMA(cm); forearm bone length, BONE(cm); heart weight, HEART(gm); spleen weight, SPLEEN(gm) and liver weight, LIVER(gm) were recorded (see Table 2) and pST-L6IXS recovered. Figure 3 represents the rate of gain (from day 0) for control (con, mean$\pm$SE, n=4) and individual values for pST-L6IXS treated pigs. Percentage weight gain, over the pST-L6IXS treatment is presented in Figure 4 with the mean$\pm$SE for control (con) pigs and individual pST-L6IXS treated animals. Plasma pST (ng/ml) was determined by radioimmunoassay (RIA) and presented in Figure 5, with mean$\pm$SE control (con) and individual concentrations for pST-L6IXS treated pigs. At slaughter the site of pST-L6IXS capsule administration was appraised (Figure 6, Plate 1, arrow) prior to removal and placement in culture media for ex-vivo assessment (Figure 6, Plate 2) of 24 hour secretion of pST (Figure 6). No apparent tissue damage or immune reactions were observed either i.m. or s.c. at day 36. However, the capsules placed in the ear (s.c.) appeared to be highly vascularised and were 100% recoverable. The capsules placed in the neck region were <10% recoverable.

The pST-L6IXS remained patent over 36 days *in vivo* and appeared to proliferate within the capsule (Plate 2) which can be removed in order to negate the concerns regarding consumption of transgenic material. Further, if the capsule is damaged (i.e. by severe tissue trauma) a normal host-graft rejection destroys the L6 cells preventing propagation of transfected material. Experiments in mice and pigs have demonstrated that pST-L6IXS are

efficacious in altering plasma pST, enhancing growth characteristics and potentially immune competence of animals.

TABLE 2

pST-L6IXS PILOT EXPERIMENT:
Pigs (male) supplied by Westmill piggery (Young, NSW)
Experiment at EMAI, maximum security piggery.
ACEC Ref No: 98/20

				LIVEWEIGHT (kg)													
				Date													
				###	##	###	##	###	###	t)/07/9n							
				Day						(slaughter)	CARCASS						
	Pen	Treatment	Animal	-14	0	7	14	21	28	36	P2us (mm)	BF (mm)	EMA (cm)	BONE (cm)	HEART (gm)	SPLEEN (gm)	LIVER (gm)
	A	con	291	24	67	NR	NR	89	95	100	11	9	54.5	24.5	388.6	159.8	1720.2
C	A	con	292	25	61	NR	NR	84	90	90	8	10	54.9	23.7	381.5	103.2	1703.6
C	B	con	294	22	74	NR	NR	94	103	104	12	15	40.5	24.4	391.5	173.2	1636.5
C	B	con	295	22	55	NR	NR	76	84	91	9	7	50.6	20.0	396.6	138.2	1561.8
T	B	3sc neck*	297	23	67	NR	NR	85	90	91	9	12	45.2	23.5	385.3	177.0	1817.7
		*infected capsule site								CvTp<0.05	nsd	nsd	CvTp<0.06	nsd	CvTp<0.05	nsd	nsd

Example 4: Pilot experiment (2) involving implantation of pST-L6IXS in pigs

A second pilot experiment was conducted in order to optimise pST-L6IXS delivery by capsules so as to achieve growth responses similar to the energy repartitioning observed with daily pST injections.

As shown in Example 1, pST secreting cells have been produced with a range of secretion rates (6-200 ng/ml). pST secretion rates in the order of 2-25 ng/ml appear to be the most stable following the imposition of stress (i.e. by bacterial contamination) on the pST secreting cells (data not shown). Accordingly, clones secreting about 5 ng/ml (clone pCI/pst-14) and about 10 ng/ml (pCI/pst-12) were selected for this pilot experiment. Male pigs (n=10, mean live weight 78.1 kg) were administered various numbers of capsules (produced according to the procedure described in Example 2) s.c. at the base of the ear (Table 3).

Pig	Capsule Number	Clone
204	1	a
216	1	b
230	3	a
202	3	b
226	5	a
206	5	b
208	10	a
224	10	b
222	100	a
228	100	b

a = clone pCI/pst- 14 (5 ng/ml)
b = clone pCI/pst-12 (10 ng/ml)

Body weights were recorded at the beginning and the end of the experiment. Animals were held in individual pens (2 m^2) and stabilised to a controlled environment facility (22^{0}C) for 1 week. The animals were offered *ad libitum* water and standard pelleted grower rations (3 kg/day @ 09:00 hrs),

and daily residues were recorded. Catheters were placed in ear veins (evc), and 24 hours later sampling commenced. Control pig (i.e. no pST capsules) blood plasma (10 ml) was collected every 30 min for 3 hours. pST capsules were administered to the ipsilateral ear immediately following serial sampling. Blood (10 ml) was collected via evc (daily @ 11:00 hrs) while catheters remained patent. Treatment (7 days post administration of pST capsules) blood plasma (10 ml) was collected every 30 min for 3 hours. Slaughter and carcass analysis was performed at about 100 kg live weight 21 days later. pST capsules were then recovered from ears and placed in *in vitro* culture (for pST assay). The capsule site was also assessed for immune responses (e.g. lymphocyte infiltration).

The results of measurements of mean (3 hr, 30 min interval) plasma pST concentration of pigs before and 7 days after receiving pST capsules (secreting between 5 and 1000 ng/ml) are shown in Figure 8. As can be seen from Figure 8, it is apparent that plasma pST is reduced in pigs following 1 week exposure to immunoneutral pST (5 - 100 ng/ml) secreting capsules.

The variability between and within individual plasma pST concentrations appeared to be more apparent during the control serial sampling period. This phenomenon is reflected in the Standard Errors about the mean observed concentrations. Further, the stable baseline and pST pulse intervals (normally 3 - 4 hrs) were not recognised by computer programs designed to identify hormone pulses. However, stable baselines and distinct pST pulses were observed in animals 1 week post pST casule administration (Figure 9).

The Rate of Gain (ROG) shown by the animals appeared to be responsive to pST capsule secretion in a dose dependent manner (Figure 10). A secretion rate of 30 ng/ml (i.e. 3 capsules secreting 10 ng/ml each) appears to be the minimum dose required to observe growth rate increases. The majority of evc's remained patent for 21 days at which time, the animals were euthanased with barbituate for carcass analysis. Analysis of carcass back fat (P2 without skin) measurements further indicate that 30 ng.ml is the minimum dose to observe energy repartitioning within 21 days of pST capsule administration (Figure 10).

Throughout the experiment there were no indications of adverse reactions, reduction in weight gain or adverse immune responses, including those animals that received 100 capsules.

Example 5: Pilot experiment (3) involving implantation of pST-L6IXS in pigs

Following example 4, investigations were conducted to assess the effect of the administering optimal pST secretion rates/capsule numbers to pigs at varying times prior to slaughter (i.e. 2, 4 and 6 weeks prior to slaughter) on back fat. 8 pigs were used for each treatment as well as 8 control (i.e. no pST capsules).

The results of the Rate of Gain measurements are provided in Figure 11.

Back fat measurements were obtained following whole carcass chilling (24 hours @ 4°C) (Figure 12). P2 measurements were recorded at the 12^{th} rib 65mm from the centre of the spinal column. Pigs exposed to capsules secreting pST for 2, 4 and 6 weeks were observed to have significantly reduced back fat. This effect in the 2 and 6 week period is approximately a 46% reduction in back fat. The animals exposed to pST IGT capsules for 4 weeks were more variable in their back fat responses, which may relate to a possible failure to recover all the capsules from a number of these animals.

Loin muscle area in pigs exposed to secreting capsules was only significantly increased (i.e. 22 %) following 6 weeks exposure to pST IGT capsules (Figure 13).

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References:

Basic *et al*, (1996) Microencapsulation and transplantation of genetically engineered cells: A new approach to somatic gene therapy. Art. Cells, Blood subs. and Immob. Biotech 24(3): 219-255.

Magari *et al*, (1997) Pharmacological control of humanised gene therapy system implanted into nude mice. J. Clin. Invest. 100: 2865-2872.

Schrader *et al*, (1996) Identification of natural monomeric response elements of the nuclear receptor R2R/ROR. They also bind to COUP-TF homodimers. J. Biol. Chem. 271:19732-19736.

Claims:

1. An expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

2. An expression cassette according to claim 1, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

3. An expression cassette according to claim 1, wherein the insulin secretory signal is a modified insulin secretory signal comprising modifications of the insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1, wherein said modifications do not deleteriously affect the biological activity of the insulin secretory signal.

4. An expression cassette according to any one of claims 1 to 3, wherein the heterologous sequence encodes a polypeptide selected from hormones, cytokines, receptor agonists, receptor antagonists, pheromones, and enzymes.

5. An expression cassette according to claim 4, wherein the polypeptide is a growth hormone.

6. An expression cassette according to claim 5, wherein the polypeptide is somatotropin.

7. An expression cassette according to any of claims 1 to 6, further including one or more regulatory elements to enable pulsatile expression of the heterologous sequence .

8. A vector including an expression cassette according to any one of claims 1 to 7.

9. A recombinant cell which includes an expression cassette according to any one of claims 1 to 7.

10. A recombinant cell according to claim 9, wherein the cell is a bacterial, yeast, insect or mammalian cell.

11. A recombinant cell according to claim 10, wherein the cell is a mammalian cell.

12. A mammalian cell according to claim 11, wherein the cell is a rat myoblast (L6) cell.

13. A method of producing a polypeptide which includes culturing a recombinant cell of any one of claims 9 to 12 under conditions enabling the expression and secretion of the polypeptide and optionally isolating the polypeptide.

14. A capsule for implantation in a host, the capsule including a semi-permeable membrane encapsulating recombinant cells according to any one of claims 9 to 12.

15. A capsule according to claim 14, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

16. A method of administering a polypeptide to a host, wherein said method includes administering to the host an expression cassette according to any one of claims 1 to 7.

17. A method of administering a polypeptide to a host, wherein the method includes implanting in the host a capsule according to claim 14 or 15.

18. A method according to claim 16 or 17, wherein the host is an animal or human.

19. A method according to claim 18, wherein the host is a livestock animal.

20. A method according to claim 19, wherein the livestock animal is a pig.

21. A method of administering somatotropin to a pig, wherein the method includes implanting in the pig a capsule including a semi-permeable membrane encapsulating recombinant cells, said recombinant cells including and expressing an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding somatotropin, wherein said membrane is permeable to the expresssed somatotropin.

22. A method according to claim 21, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

23. A method according to claim 21, wherein the insulin secretory signal is a modified insulin secretory signal comprising modifications of the insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1, wherein said modifications do not deleteriously affect the biological activity of the insulin secretory signal.

24. A method according to any one of claims 21 to 23, wherein the recombinant cells are mammalian cells.

25. A method according to claim 24, wherein the mammalian cells are rat myoblast (L6) cells.

26. A method according to any one of claims 21 to 25, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

27. A method according to any one of claims 21 to 26, wherein the pig is implanted with one or more capsules sufficient to achieve secretion of somatotropin of at least 30 ng/ml.

FIGURE 1: ISS-pST gene construct

```
   1  GCTAGCATGG CCCTGTGGAT GCGCCTCCTG CCCCTGCTGG CGCTGCTGGC
  51  CCTCTGGGGA CCTGACCCAG CCGCAGCCCT CGAGATGTTT CCAGCTATGC
 101  CACTTTCTTC TCTGTTCGCT AACGCTGTTC TTCGGGCCCA GCACCTGCAC
 151  CAACTGGCTG CCGACACCTA CAAGGAGTTT GAGCGCGCCT ACATCCCGGA
 201  GGGACAGAGG TACTCCATCC AGAACGCCCA GGCTGCCTTC TGCTTCTCGG
 251  AGACCATCCC GGCCCCCACG GGCAAGGACG AGGCCCAGCA GAGATCGGAC
 301  GTGGAGCTGC TGCGCTTCTC GCTGCTGCTC ATCCAGTCGT GGCTCGGGCC
 351  CGTGCAGTTC CTCAGCAGGG TCTTCACCAA CAGCCTGGTG TTTGGCACCT
 401  CAGACCGCGT CTACGAGAAG CTGAAGGACC TGGAGGAGGG CATCCAGGCC
 451  CTGATGCGGG AGCTGGAGGA TGGCAGCCCC CGGGCAGGAC AGATCCTCAA
 501  GCAAACCTAC GACAAATTTG ACACAAACTT GCGCAGTGAT GACGCGCTGC
 551  TTAAGAACTA CGGGCTGCTC TCCTGCTTCA AGAAGGACCT GCACAAGGCT
 601  GAGACATACC TGCGGGTCAT GAAGTGTCGC CGCTTCGTGG AGAGCAGCTG
 651  TGCCTTCTAG TCTAGA   (SEQ ID NO:4)
```

ATG...GCC- insulin secretory signal.

GCTAGC- *Nhe* I restriction site incorporated into construct in order to ligate into plasmid.

CTCGAG- *Xho* I restriction site incorporated into construct in order to ligate secretory signal and pST.

TCTAGA- *Xba* I restriction site incorporated into construct in order to ligate into plasmid.

FIGURE 2: ISS-pST peptide sequence.

```
  1  MALWMRLLPL LALLALWGPD PAAALEMFPA MPLSSLFANA VLRAQHLHQL
 51  AADTYKEFER AYIPEGQRYS IQNAQAAFCF SETIPAPTGK DEAQQRSDVE
101  LLRFSLLLIQ SWLGPVQFLS RVFTNSLVFG TSDRVYEKLK DLEEGIQALM
151  RELEDGSPRA GQILKQTYDK FDTNLRSDDA LLKNYGLLSC FKKDLHKAET
201  YLRVMKCRRF VESSCAF    (SEQ ID NO:3)
```

MAL....AAA- insulin secretory signal, cleaved upon secretion of pST.
LE- function of XhoI cleavage site; result in no predicted secondary structural changes to pST.

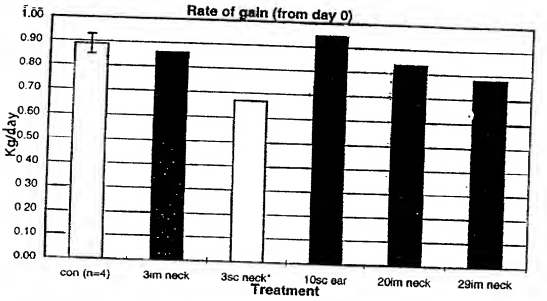

Rate of gain (from day 0)
Kg/day
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
con (n=4)
3im neck
3sc neck*
10sc ear
20im neck
29im neck
Treatment

Figure 3

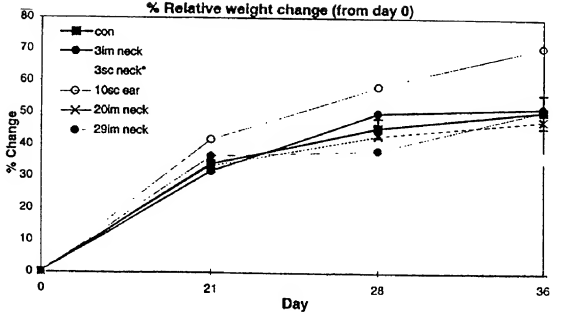
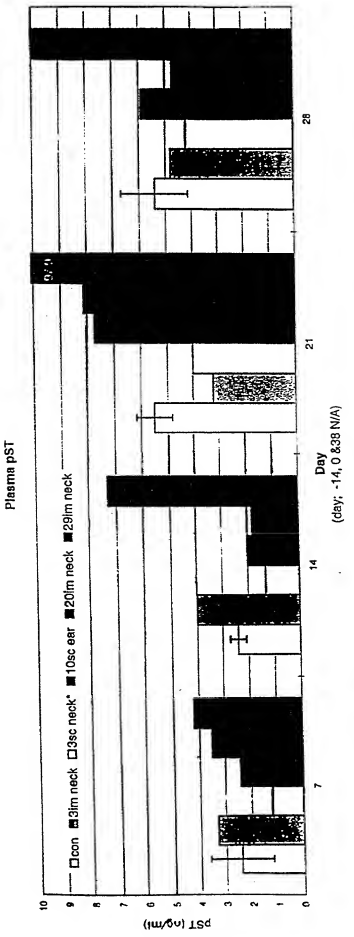

% Relative weight change (from day 0)
con
3im neck
3sc neck*
10sc ear
20im neck
29im neck
% Change
Day
0
10
20
30
40
50
60
70
80
0
21
28
36

Figure 4

Figure 5


Plasma pST
pST (ng/ml)
10
9
8
7
6
5
4
3
2
1
0
Con
3lm neck
3sc neck'
10sc ear
20lm neck
28lm neck
7
14
21
28
Day
(day: -14, 0 &38 N/A)

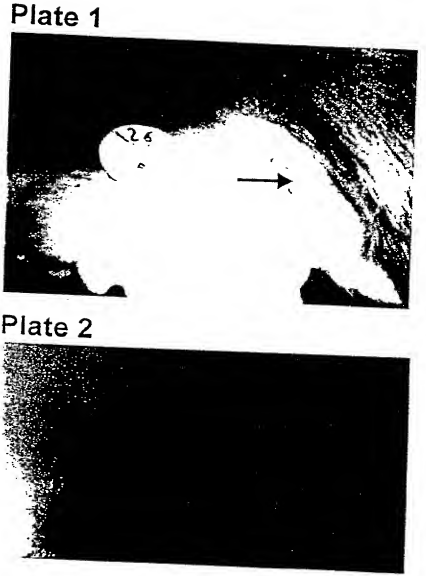


Figure 6

Figure 7

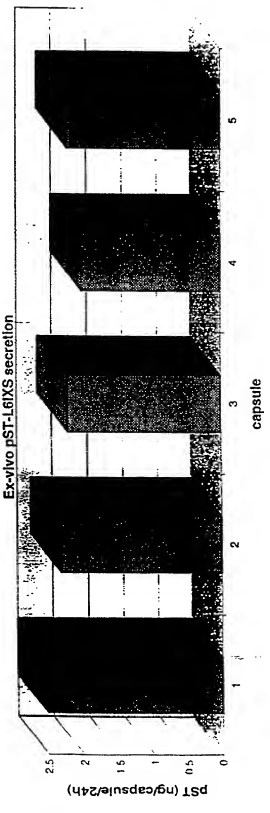

Ex-vivo pST-L6IXS secretion
pST (ng/capsule/24h)
0
0.5
1
1.5
2
2.5
1
2
3
4
5
capsule

Figure 8. Mean plasma pST (over 3 hours @ 30min intervals) before (white bars) and 1 week post pST capsule administration (black bars)(* significant).

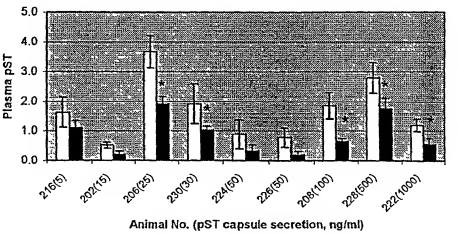


Figure 9. Daily pST concentrations

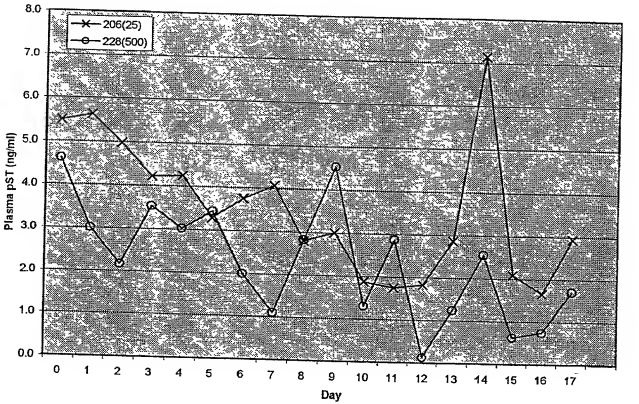


FIGURE 10

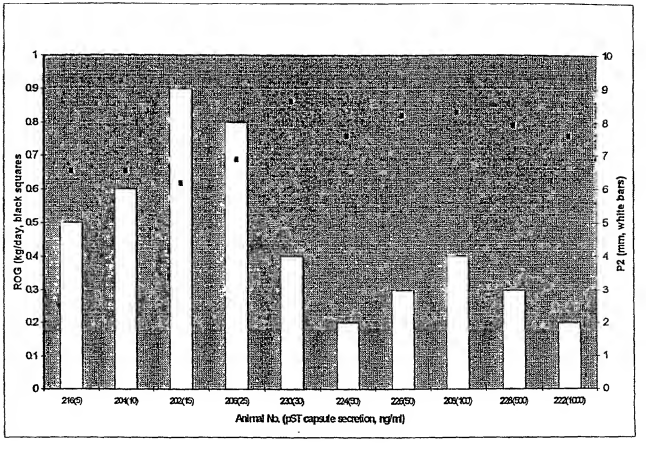

ROG (kg/day, black squares
P2 (mm, white bars)
Animal No. (pST capsule secretion, ng/ml)
216(0)
204(10)
202(15)
205(25)
233(33)
224(50)
225(50)
209(100)
228(200)
222(1000)

FIGURE 11

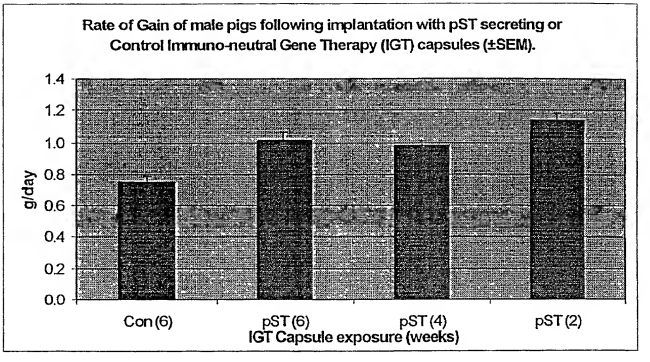

Rate of Gain of male pigs following implantation with pST secreting or Control Immuno-neutral Gene Therapy (IGT) capsules (±SEM).
g/day
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Con (6)
pST (6)
pST (4)
pST (2)
IGT Capsule exposure (weeks)

FIGURE 12

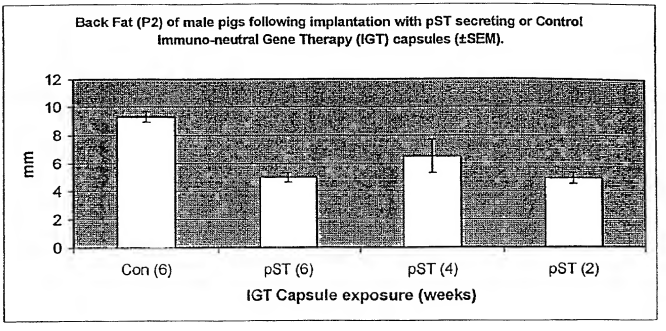
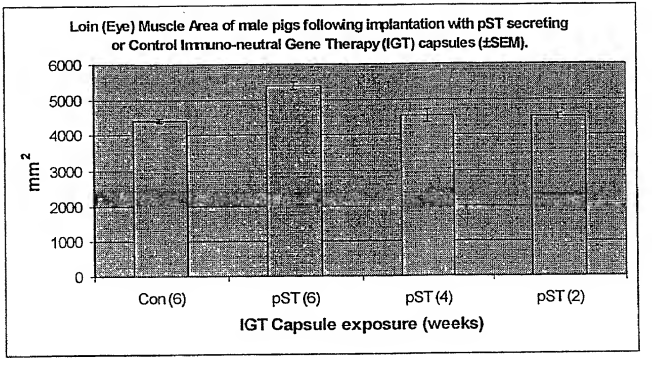


FIGURE 13



PCT/AU99/00896
Received 11 April 2000
09/807519

Sequence listing:

Applicants: Commonwealth Scientific and Industrial Research Organisation
University of Western Sydney (Nepean)
Pig Research and Development Corporation

Title of the Invention: Delivery system for porcine somatotropin

Prior Application Number: PP 6556
Prior Application Filing Date: 1998-10-16

Number of SEQ ID NOs: 4

Software: PatentIn Ver. 2.1

SEQ ID NO: 1
Length: 24
Type: PRT
Organism: Homo sapien

Sequence: 1
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15

Trp Gly Pro Asp Pro Ala Ala Ala
20

SEQ ID NO: 2
Length: 72
Type: DNA
Organism: Homo sapien

Sequence: 2

atggccctgt ggatgcgcct cctgcccctg ctggcgctgc tggccctctg gggacctgac 60
ccagccgcag cc

SEQ ID NO: 3

Length: 666

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: ISS-pST gene construct

Sequence: 3

gctagcatgg ccctgtggat gcgcctcctg cccctgctgg cgctgctggc cctctgggga 60
cctgacccag ccgcagccct cgagatgttt ccagctatgc cactttcttc tctgttcgct 120
aacgctgttc ttcgggccca gcacctgcac caactggctg ccgacaccta caaggagttt 180
gagcgcgcct acatcccgga gggacagagg tactccatcc agaacgccca ggctgccttc 240
tgcttctcgg agaccatccc ggcccccacg ggcaaggacg aggcccagca gagatcggac 300
gtggagctgc tgcgcttctc gctgctgctc atccagtcgt ggctcgggcc cgtgcagttc 360
ctcagcaggg tcttcaccaa cagcctggtg tttggcacct cagaccgcgt ctacgagaag 420
ctgaaggacc tggaggaggg catccaggcc ctgatgcggg agctggagga tggcagcccc 480
cgggcaggac agatcctcaa gcaaacctac gacaaatttg acacaaactt gcgcagtgat 540
gacgcgctgc ttaagaacta cgggctgctc tcctgcttca agaaggacct gcacaaggct 600
gagacatacc tgcgggtcat gaagtgtcgc cgcttcgtgg agagcagctg tgccttctag 660
tctaga 666

SEQ ID NO: 4

Length: 217

Type: PRT

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: ISS-pST peptide sequence

Sequence: 4

```
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1               5                  10                  15

Trp Gly Pro Asp Pro Ala Ala Ala Leu Glu Met Phe Pro Ala Met Pro
            20                  25                  30

Leu Ser Ser Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His
        35                  40                  45

Gln Leu Ala Ala Asp Thr Tyr Lys Glu Phe Glu Arg Ala Tyr Ile Pro
    50                  55                  60

Glu Gly Gln Arg Tyr Ser Ile Gln Asn Ala Gln Ala Ala Phe Cys Phe
65                  70                  75                  80

Ser Glu Thr Ile Pro Ala Pro Thr Gly Lys Asp Glu Ala Gln Gln Arg
                85                  90                  95

Ser Asp Val Glu Leu Leu Arg Phe Ser Leu Leu Leu Ile Gln Ser Trp
            100                 105                 110

Leu Gly Pro Val Gln Phe Leu Ser Arg Val Phe Thr Asn Ser Leu Val
        115                 120                 125

Phe Gly Thr Ser Asp Arg Val Tyr Glu Lys Leu Lys Asp Leu Glu Glu
    130                 135                 140

Gly Ile Gln Ala Leu Met Arg Glu Leu Glu Asp Gly Ser Pro Arg Ala
145                 150                 155                 160
```

Gly Gln Ile Leu Lys Gln Thr Tyr Asp Lys Phe Asp Thr Asn Leu Arg
165 170 175

Ser Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Ser Cys Phe Lys
180 185 190

Lys Asp Leu His Lys Ala Glu Thr Tyr Leu Arg Val Met Lys Cys Arg
195 200 205

Arg Phe Val Glu Ser Ser Cys Ala Phe
210 215

Docket No.: **(50179-088)**

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled **Delivery system for porcine somatotropin,** the specification of which
[ ] is attached hereto [X] was filed on **18 October 1999** as Application Serial No. **09/807,519** and was amended on .....(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			**Priority Claimed**	
Number	**Country**	**Day/Month/Year filed**	**Yes**	**No**
PP6556	**Australia**	**16 October 1998**	**XX**	
PCT/AU99/00896	**Australia**	**18 October 1999**	**XX**	

I hereby claim the benefit under 35 USC §119(e) of any United States provisional application(s) listed below.

Prior Provisional Application(s):

Application Number	**Filing Date**

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or Section 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

.../2

Docket No.: 50179-088 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Mitchell KEEGAN, et al.	:	
Serial No.: 09/807,519	:	Group Art Unit:
Filed: April 16, 2001	:	Examiner:

For: DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN

CORRESPONDENCE ADDRESS CHANGE

Commissioner for Patents and Trademarks
Washington, D. C. 20231

Sir:

Please change the records to indicate the current firm name and telephone number for the above-identified application and forward all future correspondence as follows:

McDERMOTT, WILL & EMERY
600 13th Street, N.W.
Washington, DC 20005-3096

202-756-8000
Facsimile: 202-756-8087

Respectfully submitted,

MCDERMOTT, WILL & EMERY

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600 13th Street, N.W.
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Date: September 17, 2001
Facsimile: (202) 756-8087

Prior U.S. Application(s):

Serial No.	Filing Date	Status: Patented, Pending, Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s): Edward A. Becker, Reg. No. 37,777; Stephen A. Becker, Reg. No. 26,527; John G. Bisbikis, Reg. No. 37,095; Kenneth L. Cage, Reg. No. 26151; Stephen C. Carlson, Reg. No. 39,929; Paul Devinsky, Reg. No. 28,553; Laura A. Donnelly, Reg. No. 38,435; Margaret M. Duncan, Reg. No. 30,879; Brian E. Ferguson, Reg. No. 36,801; Michael F. Fogarty, Reg. No. 36,139; Wilhelm F. Gadiano, Reg. No. 37,136; Keith E. George, Reg. No. 34,111; John A. Hankins, Reg. No. 32,029; Thomas A. Jolly, Reg. No. 39,241; Eric J. Kraus, Reg. No. 36,190; Edward F. Kubasiewicz, Reg. No. 30,020; Robert E. LeBlanc, Reg. No. 17,219; Jack Q. Lever, Reg. No. 28,149; Raphael V. Lupo, Reg. No. 28,363; Christine F. Martin, Reg. No. 39,762; Michael E. McCabe, Jr., Reg. No. 37,182; James H. Meadows, Reg. No. 33,965; Michael A. Messina, Reg. No. 33,424; Joseph H. Paquin, Jr., Reg. No. 31,647; Craig L. Plastrik, Reg. No. 41,254; Robert L. Price, Reg. No. 22,685; Paul A. Roberts, Reg. No. 40,289; Gene Z. Rubinson, Reg. No. 33,351; Joy Ann G. Serauskas, Reg. No. 27,952, Michele M. Schafer, Reg. No. 34,717; David J. Serbin, Reg. No. 30,589; Glenn Snyder, Reg. No. 41,428; Arthur J. Steiner, Reg. No. 26,106; David L. Stewart, Reg. No. 37,578; Leonid D. Thenor, Reg. No. 39,397; Keith J. Townsend, Reg. No. 40,358; Leon R. Turkevich, Reg. No. 34,035; Christopher D. Ward, Reg. No. 41,367; Damian G. Wasserbauer, Reg. No. 34,749; Aaron Weisstuch, Reg. no. P41,557; Edward J. Wise, Reg. No. 34,523; Alexander V. Yampolsky, Reg. No. 36,324; and Robert W. Zelnick, Reg. No. 36,976 all of

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with full power of substitution and revocation, to prosecute this application and to transact all business in the patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

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Inventor's signature: Date: 13.07.01

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